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Effect of Nano-Phytosome of Quercetin on Mice Liver Infected with *Plasmodium berghei*

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ABSTRACT

Aims Malaria is one of most important diseases especially in tropical regions. Flavonoids are known to have beneficial properties that could be profitable. This study aimed to evaluate the effects of nano-phytosomes of Quercetin on liver damages of mice infected with *Plasmodium berghei*.

Materials & Methods A total of 60 male BALB/c mice were intra-peritoneally infected by administration of 106 *P. berghei*-infected RBCs. Animals were acclimatized for 7 days and divided into 5 groups; 0.9% isotonic saline (as negative control), saline treated (as positive control), 2mg/kg of Hydroxychloroquine sulfate treated for 4 days (HF), 10mg/kg of nano-phytosomes of Quercetin treated for 4 days (NQ), and 10mg/kg of nano-phytosomes of Quercetin + 2mg/kg of Hydroxychloroquine sulfate for 4 days (NQ+HF). Histo-pathological parameters and pro-inflammatory cytokines were evaluated.

Findings Administration of *P. berghei* could increase the scores for histo-pathological parameters and levels of pro-inflammatory cytokines ($p<0.0001$). Administration of Hydroxychloroquine sulfate and nano-phytosomes of Quercetin could alleviate adverse effects of *P. berghei* on histo-pathological parameters ($p<0.05$).

Conclusion A combination of nano-phytosomes of Quercetin and Hydroxychloroquine sulfate shows the best effect on treatment of *Plasmodium berghei* infections.

Keywords Malaria; Pro-Inflammatory Cytokines; Nano-Phytosomes; Quercetin; Liver Damages

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[1] Antimicrobial resistance global report on surveillance: 2014 Summary [2] Diarrhea caused by primarily non- gastrointestinal infections [3] Histopathologic study on mice infected with Plasmodium berghei after treatment with sulfadoxine- pyrimethamine, pyrimethamine, and hydroxychloroquine sulfate [4] Immunological processes in malaria pathogenesis [5] A novel approach towards phytosomal flavonoids [6] Flavonoids, a class of natural products of high pharmacological potency [7] Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man [8] Anti- inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat [9] Screening of flavonoid "quercetin" from the rhizome of Smilax china Linn. for anti- psoriatic activity [10] Quercetin, a flavonoid with anti-inflammatory activity, suppresses the development of abdominal aortic aneurysms in mice [11] Preparation and characterization of lipid based nanosystems for topical delivery of quercetin [12] Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles [13] Quercetin deformable liposome: Preparation and efficacy against ultraviolet B induced skin damages in vitro and in vivo [14] Phytic acid enhances the oral absorption of isorhamnetin, quercetin, and kaempferol in total flavones of Hippophae rhamnoides L [15] Prooxidant activities of quercetin, p- courmaric acid and their derivatives analysed by quantitative structure- activity relationship [16] Phytosomes: An emerging trend in herbal drug treatment [17] Treatment of pregnant BALB/c mice with sulphadoxine pyrimethamine or chloroquine abrogates Plasmodium berghei induced placental pathology [18] Medical parasitology Austin [19] Role of medicinal plants in wound healing [20] Ameliorative effect of dietary genistein on diabetes induced hyper-inflammation and oxidative stress during early stage of wound healing in alloxan induced diabetic mice

Introduction

Malaria is one of most important diseases especially in tropical regions and it is a big challenge for most people in all over world. Plasmodium spp. are main factor for malaria. Organization like WHO has estimated to be 200 million people involved with malaria [1].

The different factors such as parasite growth, proliferation, and ability of host immune responses could influence pathogenesis of malaria [2]. The different symptoms has been reported for patients involved with malaria including ague, anemia and cerebral and kidney disorders and splenomegaly [3].

It has been accepted to use animal model for study of animals due to limitations for nature and interpretation under in vitro conditions. *Plasmodium berghei* is known to have specific characteristics and red blood cell (RBC) tropisms have been used as experimental models in human model [4]. Liver is site for infection of malaria and is involved in the diseases. Inflammatory parameters can be used as markers assessment of liver damages.

Some synthetic agents have commonly been used for treatment of malaria such as chloroquine, pyrimethamine, and sulfadoxine-pyrimethamine. There is increasing interest for application of natural agents for treatment of diseases and/or as adjuvant therapy. Natural anti-inflammatory agents may protect patients with malaria from inflammation.

In this connection, the Flavonoids are known to have beneficial properties including antioxidant, antimicrobial, anticancer and anti-inflammatory [5].

Quercetin is a one of polyphenolic flavonoids which is found in fruits, vegetables and herbs and is used for health benefits such as antioxidant, anti-inflammatory and antimicrobial activities [6, 7]. It is known to have major limitations in bioavailability and absorption [8-10]. Inefficiency of absorption could be attributed to inappropriate lipid solubility, big size of molecules that cannot be absorbed through passive diffusion in intestine-bloodstream pathway, and destroy of phenol moiety of Quercetin by bacteria in gastric space [11-14]. It is essential to use a noval carrier for Quercetin [15]. Phytosome is one novel delivery system that could improve absorption and bioavailability of phyto constituent [16].

It seems that Quercetin could protect liver from damages and inflammation by its antioxidant and anti-inflammatory properties. This novel study aimed to evaluate the application of Nano-phytosomes of Quercetin (NQ) as main agent and/or adjuvant therapy for treatment of infection induced by *Plasmodium berghei* in mice model., in cases where the doctor is not sure of complete reduction, radiography is recommended for control.

Also, in these studies following reduction, even in cases with fractures, no significant new fractures were seen in control radiography [15].

Due to the fact that radiography before and after reduction requires significant waste of time, the patient is exposed to harmful radiation and imposes a significant material burden on patients, the present study was conducted based on these objectives to see if the physician's clinical judgment in determining the presence of anterior shoulder dislocation is so accurate that preoperative radiography can be ruled out in these patients. Positive results, improvements in the process of diagnosis and treatment of these patients.

Materials and Methods

Preparation of NQ

Thin layer hydration procedure was used to prepare the NQ. Quercetin (Sigma Aldrich; Germany) and soybean phosphatidylcholine (Merck; Germany) were firstly dissolved in methanol (Merck; Germany), but cholesterol (Merck; Germany) was dissolved in dichloromethane (Merck; Germany). The resulted mixture was kept in a flask.

To evaporate the mixture, a rotary evaporator was used and all the solvents were kept to produce a thin dry film. The prepared lipid thin layer had been exposed to nitrogen gas flow and maintained in an overnight and in the room temperature before hydration to ensure the complete removal of the organic solvents. The film was hydrated with distilled water in a rotary at 45°C. Several procedures were applied to reduce the phytosomes size such as bath sonication (Model 8852, Cole-Palmer; USA) in 45°C, homogenization (Heidolph; Germany) with 20,000rpm and probe-sonication method (Sonics VCX 750; USA).

Animals and Bacteria

A total of 60 male BALB/c mice, 10-12 weeks of age and initial weight 27±3g, were used. The mice were intra-peritoneally infected by administration of 106 *P. berghei*-infected RBCs.

Hydroxychloroquine sulfate (Biogen; India) was used as recommended by WHO in a dose of 2mg/kg body weight daily for 4 days. Mice were acclimatized for 7 days and divided into 5 groups:

1) Mice received 0.9% isotonic saline and considered as negative control (NC);

2) Non-treated infected mice that were considered as positive control (PC);

3) Infected mice treated with 2mg/kg body weight of HF for 4 days (HF);

4) Infected mice treated with 10 mg/kg body weight of NQ for 4 days (NQ); and

5) Infected mice treated with 10mg/kg body weight of NQ and 2 mg/kg body weight of HF for 4 days (NQ+HF).

A schematic design of study is presented in Figure 1.

Histological study and pro-inflammatory cytokines

In day 28, following scarification, liver was separated and fixed in 10% neutral buffered formalin. Samples were inserted into paraffin and histological changes were evaluated. Scores were considered in scale of 0 up to 4, including not observed (0), very mild (1), mild (2), moderate (3), and severe (4). Blood samples were collected and tumor necrosis factor (TNF)-α and interleukin (IL)-1β levels in the serum were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits.

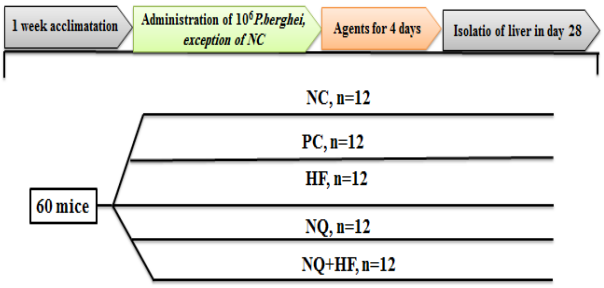

Figure 1. Experimental design and groups

Statistical analyses

The data were reported as mean±standard deviation. One-way ANOVA with Tukey's post hoc tests were applied to compare among groups.

Findings

Histo-pathological parameters

Administration of *P. berghei* could increase scores for histo-pathological parameters in terms of kupffer cell hyperplasia, hemosiderosis, hepatic necrosis and periportal inflammation in comparison to NC and PC groups ($p<0.0001$; Figure 2). Administration of HF and NQ could alleviate adverse effects of *P. berghei* on histo-pathological parameters ($p<0.05$). The responses were better in HF in comparison to NQ ($p<0.05$). A combination of NQ and HF could have better effects on histo-pathological parameters in comparison to single form ($p<0.05$).

Pro-inflammatory cytokines

The data for pro-inflammatory cytokines are shown in Figure 3.

Figure 2. Effects of experimental treatments on histo-pathological parameters of mice involved with malaria (Significant difference between NC group and other groups in 0.05*, 0.01**, 0.001***, and 0.0001****; Significant difference between PC group and other groups in 0.05+, 0.01++, 0.001+++, and 0.0001++++)

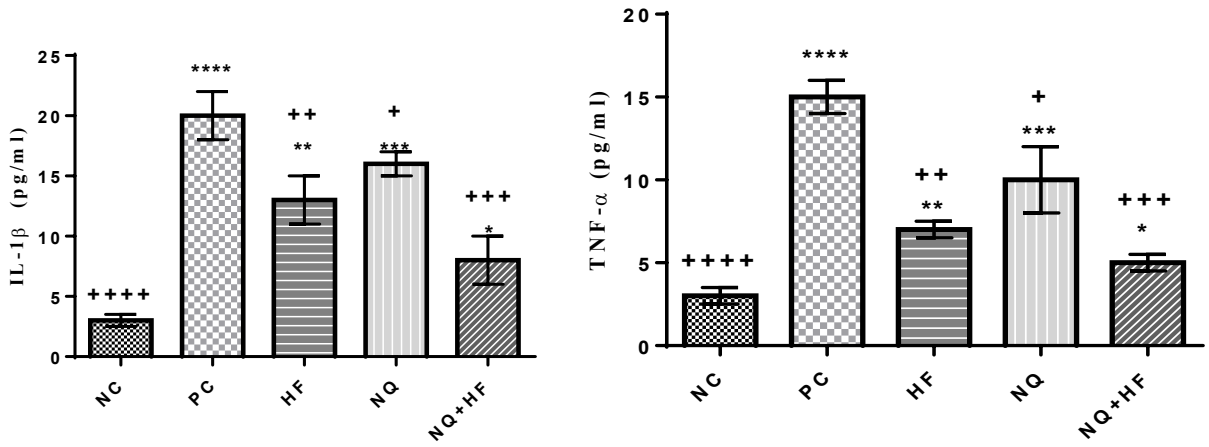

Figure 3. Effects of experimental treatments on serum pro-inflammatory cytokines of mice involved with malaria (Significant difference between NC group and other groups in 0.05*, 0.01**, 0.001***, and 0.0001****; Significant difference between PC group and other groups in 0.05+, 0.01++, 0.001+++, and 0.0001++++)

The levels of inflammatory cytokines were significantly higher in PC group in comparison to NC group ($p<0.0001$). Administration of NQ, HF and specially their combination could significantly decrease levels of pro-inflammatory cytokines ($p<0.05$).

Discussion

Malaria is a disease that needs more attentions because it is not still exterminated and the best control procedure is application of a true treatment procedure. Anti-malarial drugs have been used for treatment of malaria but it is essential to investigate their toxicity and damage [17]. The resulted findings for histo-pathological parameters in positive group show that proliferation of the parasites in the blood. As malaria progresses, hepatocytes are destroyed and merozoites are transferred into the blood circulation. Our findings for cytokines confirm such claim. Our findings for kupffer cell hyperplasia showed to be lower in the treated groups and had difference with PC group; showing that the agents could prevent proliferation of liver macrophages. Following the rupture the RBCs, hemoglobin splitting is done and hemosiderosis occurs [18]. In the present study HF and NQ, especially in combination form could alleviate the adverse effects of malaria. The Flavonoids are known to have most common group of polyphenolic compounds and have antioxidant, antimicrobial and anti-inflammatory properties [5].

In summary, NQ showed better activity in combination with HF. NQ has low water solubility and its application in nano-phytosom form could improve solubility and help more absorption. Activated host immune system increases fight against infection, whereas overproduced inflammation causes to produce the tissue damage or even multiple organ failure. It has been shown that the flavonoids and triterpenoids may encourage to treat that could be attributed to antimicrobial properties [19]. Nuclear factor-kappa B (NF-κB) is one mediator for inflammatory response which increases inflammatory cytokines including TNF-α and IL-1β [20]. These findings suggest that NQ does not have effects such as HF, but in combination with HF could increase its efficiency. Future studies are needed to evaluate the effects of NQ on improvement of conditions in mice with malaria, but our findings suggest that NQ can be used as adjuvant therapy in treatment of malaria.

Conclusion

Nano-phytosomes of Quercetin can alleviate adverse effects of malaria in terms of liver damages and inflammation.

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